# THE ANTIBIOTIC BEHAVIOR

# OF

# CANAMIN CLAY

by

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Degree of Bachelor of Science
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Signature of Faculty Advisior

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Date: May 18, 1951

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May 18, 1951

Professor Joseph S. Newell
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Cambridge, Massachusetts

Dear Sir:

In partial fulfillment of the requirements for the degree of Bachelor of Science, I submit herewith my thesis, entitled "The Antibiotic Behavior of Canamin Clay".

Respectfully yours,

## ACKNOWLEDGMENTS

The author wishes to express her deepest appreciation and sincere gratitude:

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## SUMMARY

The purpose of this work was to ascertain the cause and the degree of the inhibition of bacterial growth exhibited by Canamin clay. Screening tests for antibiotics and adsorbtion tests with bacteria were conducted under varying conditions. The Agar Diffusion, Agar Plate Dilution and the Serial Dilution procedures were modified slightly for use with the clay and four of its fractions.

No inhibition of bacterial growth could be observed in these tests. It is recommended that an investigation of the clay be continued with specific reference to its properties as a protective coating for the infected area.

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# INTRODUCTION

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The discovery of Canamin clay and its subsequent use in the healing of epidermal and intestinal infections resulted in basic research in order to determine the cause of this healing ability. Work had been done up to December 1950 on the physical and chemical nature of the clay. It had been discovered that the clay was an aluminum silicate of varying particle size and that the percent of elements present varied with the particle size. The smallest particle size could not be photographed clearly under the electron-microscope.(1).

The very high ratio of surface area to volume in the clay led to the hypothesis that the clay owed its healing properties to the adsorbtion of bacteria and the inhibition of the bacteria growth because of it. The bacteria were observed to be adsorbed by the clay under the ultramicroscope.(1).

The problem remained as to whether the adsorbtion of the bacteria affected their growth, and if so, which fractions of the clay were most effective in adsorbing the bacteria. The purpose of this thesis was to investigate the above problems, and also to determine the effectiveness of the clay when used with Penicillin.

It was decided to use the general tests for antibiotics, and to adapt these methods to the study of adsorbtion on various fractions of the clay. Consideration of the list of important variables:

1. the method of bringing the bacteria into contact with the clay,

2. the physical and chemical nature of the medium,

3. the type of bacteria, and

4. the type of control led to the conclusion that more than one experimental method should be used.

The standard assay method for antibiotics was the first scheme selected.(4). However, the bacteria and the clay are separated by porcelain cylinders. Due to the unknomn effect of the cylinders this scheme was abandoned.

Three of the screening tests were selected from Waksman's techniques. (4,6). They had different ways of bringing the bacteria into contact with the clay. In the Agar Diffusion method the bacteria and clay were not in contact. This was done to eliminate the possibility of other causes for the behavior of the clay. In the Agar Plate Dilution method the contact was made in the presence of agar, and in the Serial Dilution method the contact was made in the presence of nutrient broth.

# PROCEDURE

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A. Preparation of Solutions

The Canamin clay was centrifuged to obtain the three fractions used. A one percent solution of the clay in distilled water was passed through the supercentrifuge until about three liters of overflow had been collected. The top one-quarter of the celluloid was selected as the fine fraction. The middle fraction was selected as a strip about one half inch in width at the center portion where no obvious discontinuity in particle size occured. The coarse fraction was removed from the lower half inch of the cellulose strip.

This procedure was repeated until fifteen grams of the fine fraction were collected. The middle and coarse fractions were both available in larger quantities.

In the Agar Diffusion and the Agar Plate-Dilution methods the sample of clay and each of the samples of clay fractions were dispersed in sterile distilled water by shaking them on the agar-agar shaker for five or six hours before preceding with the experiments.

In the Agar Plate-Dilution method the clay dispersions were sterilized in an autoclave for fifteen minutes at fifteen pounds pressure.

In the Serial Dilution method a one gram sample and a two gram sample of the superfine Canamin clay were sterilized in an autoclave for fifteen minutes at fifteen pounds pressure.

2. Bacteria

The test organisms of E. Coli, Bacillus s and Staph. aureus were incubated for 24 hours at 37 C.

3. Penicillin

Crystalline Penicillin G Sodium was dissolved with sterile distilled water to dilutions of 10,000, 1,000, 10, and 1, units / ml.

B. Waksman's Cylinder Plate Method

Nutrient agar was added to the sterile Petri dishes to an approximate depth of 4mm. To the still molten agar was added 3 ml. of a 1:10 dilution of the Staphylococcus aureus. The Petri dish was then placed in the incubator at 37°C for 2 hours with the cover resting on three 3x5 mm porcelain cylinders. The purpose of this last step was to remove excess water. Five sterile porcelain cylinders of the same dimensions were dropped from a height of 1/8 of an inch into the agar surface. The cylinders were equidistant from the center of the Petri dish.

The cylinders were filled in order with:

1. the 10 unit / ml. solution of penicillin.
2. the concentrated suspension of one fraction of the clay.
3. the one unit / ml. solution of penicillin.
4. the 1:10 solution of the same fraction of clay.
5. the 1:100 solution of the same fraction of clay.

The Petri dishes were incubated at 37°C for 24 hours, and then examined for zones of inhibition. This method was abandoned because of the unknown effect of the porcelain cylinders on the clay.

C. Agar Diffusion Method

Nutrient agar was added to sterile Petri dishes to a depth of 4 mm. The agar was seeded with one ml. of the test organism and then allowed to harden. A sterile cork borer, size 15, and a sterile spatula were used to remove the center of the agar. The space between the bottom of the plate and the agar was sealed by pipetting one ml. of agar into the hole and allowing it to harden. One ml. of the test or control sample was pipetted into the hole.

The plates were incubated for fifteen hours at 37° C and examined for evidences of bacterial growth. The width of the clear ring of agar was measured.

D. Agar Plate-Dilution

One ml. of the test or control sample was added to the Petri dish. Nutrient agar was added, and carefully mixed with the sample. The agar was allowed to solidfy and streaked with the test organism. The plates were incubated for 24 hours at 37° C and examined for bacterial growth. Slides were made from the growth taken from the Petri dishes containing the more concentrated samples. This was done to verify the visual observations.

E. The Serial Dilution Method

A sterile one gram sample and a sterile two gram sample of superfine Canamin clay were added to different test tubes, each containing 9 ml. of nutrient broth. The test tubes were shaken for three hours on the agar-agar shaker. One ml. of each of these dispersions was diluted with 9 ml. of nutrient broth.

One ml. of Staphylococcus Aureus was added to each of the four test tubes and they were again shaken for about a half hour. Two one ml. portions from each test tube were added to different test tubes containing 9 ml. of the nutrient broth. All test tubes were incubated for 24 hours at 37° C. Slides were made from each test tube.

After twenty-four hours of incubation, two one ml. portions from each test tube were again added to separate test tubes containing 9 ml. of nutrient broth. These test tubes were incubated for 24 hours at 37 C. Slides were made from each test tube.

F. Controls

In each experiment a similar run was conducted using no clay. These blanks were only one form of control used. Penicillin was used as a control with gram positive bacteria, ie., Staph. Aureus, and Bacillus s.

# RESULTS

## RESULTS

In testing the Canamin clay for antibiotic behavior, no inhibition of the bacterial growth could be observed by the Agar Diffusion or the Agar Plate Dilution methods. No inhibition of the bacterial growth was observed when the clay was tested for its adsorbtive properties by the Serial Dilution method. While the clay does adsorb some of the bacteria, the bacterial growth is not noticeably retarded by this adsorbtion.

The failure of the clay to show any inhibition of bacterial growth eliminated the possibilities of discovering the relative effectiveness on bacterial growth of the various fractions, and their effectiveness when used jointly with Penicillin.

# CONCLUSIONS

# AND

# RECOMMENDATIONS

## CONCLUSIONS AND RECOMMENDATIONS

As a result of this work it is concluded that the adsorbtive properties of Canamin clay do not cause inhibition of bacterial growth, and that the Canamin clay does not possess any antibiotic behavior.

It is recommended that the healing of infections by Canamin clay be further investigated. The properties of the clay as a protective coating for the infections is postulated as one possible means by which the healing takes place.

# APPENDIX

Table I

Method: Agar Diffusion

Bacteria: Staphylococcus aureus

Medium: nutrient agar

Incubation temp.: 37°C

Incubation period: 15 hours

Clay: Canamin clay and its fractions not sterilized

| Plate Number | Clay Fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | Canamin clay | concentrated | no inhibition |
| 2 | Canamin clay | 1:10 | no inhibition |
| 3 | Canamin clay | 1:100 | no inhibition |
| 4 | fine | concentrated | no inhibition |
| 5 | fine | 1:10 | no inhibition |
| 6 | fine | 1:100 | no inhibition |
| 7 | middle | concentrated | no inhibition |
| 8 | coarse | concentrated | no inhibition |
| 9 | no clay | --------- | bacterial growth |

| Plate Number | Penicillin concentration | Clay | Observations |
|---|---|---|---|
| 10 | 10 units / ml. | ------ | inhibition zone-3/8 in. |
| 11 | 1 unit / ml. | ------ | inhibition zone -1/16 in. |
| 12 | 10 units / ml. | Canamin clay | inhibition zone -3/8 in. |
| 13 | 1 unit / ml. | Canamin clay | inhibition zone- 1/16 in |
| 14 | 1 unit / ml. | fine fraction | inhibition zone- 1/16 in. |

TABLE II

Method: Agar Diffusion

Bacteria: Escherichia coli

Medium: nutrient agar

Incubation temp.: 37 °C

Incubation period: 15 hours

Clay: Canamin clay and its fractions not sterilized

| Plate Number | Clay Fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | Canamin clay | concentrated | no inhibition |
| 2 | Canamin clay | 1:10 | no inhibition |
| 3 | Canamin clay | 1:100 | no inhibition |
| 4 | fine | concentrated | no inhibition |
| 5 | fine | 1:10 | no inhibition |
| 6 | fine | 1:100 | no inhibition |
| 7 | middle | concentrated | no inhibition |
| 8 | coarse | concentrated | no inhibition |
| 9 | no clay | ----- | bacterial growth |

Table III

Method: Agar Diffusion

Bacteria: Bacillus subtilis

Medium: nutrient agar

Incubation temp.: 37°C

Incubation period: 15 hours

Clay: Canamin clay and its fractions not sterilized

| Plate number | Clay Fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | Canamin clay | concentrated | no inhibition |
| 2 | Canamin clay | 1:10 | no inhibition |
| 3 | Canamin clay | 1:100 | no inhibition |
| 4 | fine | concentrated | no inhibition |
| 5 | fine | 1:10 | no inhibition |
| 6 | fine | 1:100 | no inhibition |
| 7 | middle | concentrated | no inhibition |
| 8 | coarse | concentrated | no inhibition |
| 9 | no clay | ----- | bacterial growth |

| Plate number | Penicillin concentration | Clay | Observations |
|---|---|---|---|
| 10 | 10 units/ ml. | ------ | inhibition zone-3/8 in. |
| 11 | 1 unit / ml. | ------ | inhibition zone -1/16 in. |
| 12 | 10 units / ml. | Canamin clay | inhibition zone -3/8 in. |
| 13 | 1 unit / ml. | Canamin clay | inhibition zone- 1/16 in. |
| 14 | 1 unit / ml. | fine fraction | inhibition zone - 1/16 in. |

TABLE IV

Method: Agar Plate- Dilution

Bacteria: Staphylococcus aureus

Medium: nutrient agar

Incubation temp.: 37° C

Incubation period: 24 hours

Clay: sterile

| Plate Number | Clay fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | --------- | ------- | bacterial growth |
| 2 | Canamin clay | concentrated | no inhibition |
| 3 | Canamin clay | concentrated | no inhibition |
| 4 | Canamin clay | 1:10 | no inhibition |
| 5 | Canamin clay | 1:100 | no inhibition |
| 6 | fine fraction | concentrated | no inhibition |
| 7 | fine fraction | concentrated | no inhibition |
| 8 | fine fraction | 1:10 | no inhibition |
| 9 | fine fraction | 1:100 | no inhibition |
| 10 | middle fraction | concentrated | no inhibition |
| 11 | coarse fraction | concentrated | no inhibition |

TABLE IV (con't)

| Plate Number | Penicillin Concentration | Clay | Observations |
|---|---|---|---|
| 12 | 10,000 units /ml. | ----- | clear |
| 13 | 10,000 units/ml. | ------ | clear |
| 14 | 1,000 units/ml. | ------- | clear |
| 15 | 1,000 units / ml. | ------- | clear |
| 16 | 10,000 units /ml. | Canamin clay | clear |
| 17 | 10,000 units/ml. | Canamin clay | clear |
| 18 | 10,000 units /ml. | Canamin clay | clear |
| 19 | 1,000 units /ml. | Canamin clay | clear |
| 20 | 1,000 units /ml. | Canamin clay | clear |
| 21 | 1,000 units /ml. | Canamin clay | clear |

TABLE V

Method: Agar Plate- Dilution

Bacteria: Escherichia coli

Medium : nutrient agar

Incubation temp.: 37°C

Incubation period: 24 hours

| Plate Number | Clay Fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | ------- | ------- | bacterial growth |
| 2 | Canamin clay | concentrated | no inhibition |
| 3 | Canamin clay | concentrated | no inhibition |
| 4 | Canamin clay | 1:10 | no inhibition |
| 5 | Canamin clay | 1:100 | no inhibition |
| 6 | fine fraction | concentrated | no inhibition |
| 7 | fine fraction | concentrated | no inhibition |
| 8 | fine fraction | 1:10 | no inhibition |
| 9 | fine fraction | 1:100 | no inhibition |
| 10 | middle fraction | concentrated | no inhibition |
| 11 | coarse fraction | concentrated | no inhibition |

TABLE VI

Method: Agar Plate-Dilution

Bacteria: Bacillus subtilis

Medium: nutrient agar

Incubation temp.: 37°C

Incubation period: 24 hours

Clay: sterile

| Plate Number | Clay fraction | Dilution of clay | Observations |
|---|---|---|---|
| 1 | ------ | ----- | bacterial growth |
| 2 | Canamin clay | concentrated | no inhibition |
| 3 | Canamin clay | concentrated | no inhibition |
| 4 | Canamin clay | 1:10 | no inhibition |
| 5 | Canamin clay | 1:100 | no inhibition |
| 6 | fine fraction | concentrated | no inhibition |
| 7 | fine fraction | concentrated | no inhibition |
| 8 | fine fraction | 1:10 | no inhibition |
| 9 | fine fraction | 1:100 | no inhibition |
| 10 | middle fraction | concentrated | no inhibition |
| 11 | coarse fraction | concentrated | no inhibition |

TABLE VI (con't)

| Plate number | Penicillin concentration | Clay | Observation |
|---|---|---|---|
| 12 | 10,000 units/ml. | --- | clear |
| 13 | 10,000 units/ml. | --- | clear |
| 14 | 1,000 units/ml | --- | clear |
| 15 | 1,000 units/ml. | --- | clear |
| 16 | 10,000 units/ml. | Canamin clay | clear |
| 17 | 10,000 units/ml. | Canamin clay | clear |
| 18 | 10,000 units/ml. | Canamin clay | clear |
| 19 | 1,000 units/ml. | Canamin clay | clear |
| 20 | 1,000 units/ml. | Canamin clay | clear |
| 21 | 1,000 units/ml. | Canamin clay | clear |

TABLE VII

Method: Serial Dilution

Bacteria: Staphylococcus aureus

Medium: Nutrient broth

Incubation period: 24 hours

Incubation temp: 37°C

Clay: sterilized superfine Canamin clay

| Tube number | Clay Dilution (gm. /ml/ broth) | Comments | Observations under microscope |
|---|---|---|---|
| 1 | .2 | original dispersion | large bacteria agglomerates |
| 2 | .1 | original dispersion | large bacteria agglomerates |
| 3 | .018 | diluted from no. 1 | bacteria agglomerates |
| 4 | .009 | diluted from no. 2 | bacteria agglomerates |
| 5 | -------- | control | bacterial growth |
| 6 | .02 | diluted from no. 1 | no inhibition |
| 7 | .02 | diluted from no.1 | no inhibition |
| 8 | .01 | diluted from no. 2 | no inhibition |
| 9 | .01 | diluted from no.2 | no inhibition |
| 10 | .002 | diluted from no.3 | no inhibition |
| 11 | .002 | diluted from no.3 | no inhibition |
| 12 | ----- | diluted from no 5 | bacterial growth |

Table VII (con't)

| Tube number | Clay Dilution (gm./ml. broth) | Comments | Observations under microscope |
|---|---|---|---|
| 13 | .001 | diluted from no. 4 | no inhibition |
| 14 | .001 | diluted from no.4 | no inhibition |
| 15 | .02 | diluted from no. 1 after 24 hours | no inhibition |
| 16 | .02 | diluted from no. 1 after 24 hours | no inhibition |
| 17 | .01 | diluted from no. 2 after 24 hours | no inhibition |
| 18 | .01 | diluted from no.2 after 24 hours | no inhibition |
| 19 | .002 | diluted from no. 3 after 24 hours | no inhibition |
| 20 | .002 | diluted from no. 3 after 24 hours | no inhibition |
| 21 | .001 | diluted from no. 4 after 24 hours | no inhibition |
| 22 | .001 | diluted from no. 4 after 24 hours | no inhibition |
| 23 | ------ | diluted from no.5 after 24 hours | bacterial growth |

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